Influence of Spider Silk on Refugia Preferences of the Recluse Spiders *Loxosceles reclusa* and *Loxosceles laeta* (Araneae: Sicariidae)

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ABSTRACT In a previous experimental study, recluse spiders *Loxosceles reclusa* Gertsch and Mulaik and *Loxosceles laeta* (Nicolet) (Araneae: Sicariidae) preferred small cardboard refugia covered with conspecific silk compared with never-occupied refugia. Herein, we investigated some factors that might be responsible for this preference using similar cardboard refugia. When the two *Loxosceles* species were given choices between refugia previously occupied by their own and by the congeneric species, neither showed a species-specific preference; however, each chose refugia coated with conspecific silk rather than those previously inhabited by a distantly related cribellate spider, *Metaltella simoni* (Keyserling). When *L. laeta* spiders were offered refugia that were freshly removed from silk donors compared with heated, aged refugia from the same silk donor, older refugia were preferred. Solvent extracts of *L. laeta* silk were chosen approximately as often as control refugia when a range of solvents (methylene chloride:methanol, water, and hexane) were used. However, when acetone was used on similar silk, there was a statistical preference for the control, indicating that there might be a mildly repellent aspect to acetone-washed silk. Considering the inability to show attraction to chemical aspects of fresh silk, it seems that physical attributes may be more important for selection and that there might be repellency to silk of a recently vacated spider. These findings are discussed in regard to pest management strategies to control recluse spiders.

KEY WORDS brown recluse spider, *Loxosceles*, Arachnida, sericophily, urban entomology

Spiders use silk cues for a variety of assessments about their environment. Most research has focused on reproductive behavior where a diverse array of spiders use both chemical and physical signals to determine the location, reproductive status, and receptivity of mates (Ross and Smith 1979, Suter and Renkes 1982, Roland and Rovner 1983, Jackson 1986, Suter and Hirscheimer 1986, Lizotte and Rovner 1989, Anava and Lubin 1993, Clark and Jackson 1995, Prouvost et al. 1999, Tichy et al. 2001, Gaskett et al. 2004, Roberts and Uetz 2005, Hoefler 2007). Yet, despite a modest amount of research regarding chemical communication in arachnids, sex pheromones have been identified in only five spider species: *Cupiennius salei* (Keyserling) (Ctenidae), *Linyphia triangularis* (Clerck) (Linyphiidae), *Agelenopsis aperta* (Gertsch) (Agelenidae), *Argiope bruennichi* (Scopoli), and *Latrodectus hasselti* Thorell (Gaskett 2007, Chinta et al. 2010, Jerhot et al. 2010). Sometimes, only a single strand of odor-laden silk is required to elicit mate-seeking behavior (Rovner and Barth 1981, Roland 1984). Silk also can provide qualitative information regarding nonsexual interactions such as the risk of predation from conspecifics (Clark et al. 1999), the presence of conspecific rivals (Clark and Jackson 1995), assessment of one's own web and that of siblings (sibs) and nonsibs in a philopatric species (Bilde et al. 2002), and website attractiveness (Hodge and Storfer-Isser 1997, Leborgne and Pasquet 1987). These chemical mediators vary from those that are degraded by water (wolf spiders) (Dondale and Hegdekar 1973) to those where water has no detrimental effect (fishing spiders) (Roland and Rovner 1983).

Loxosceles spiders, commonly known as recluse or violin spiders, are infamous worldwide for causing necrotic skin lesions in humans, although this propensity is often the fodder for fanciful exaggeration (Vetter 2008). They are not social spiders by the strictest definition; however, populations can reach hundreds to thousands of spiders per home (Schenone et al. 1970, Vetter and Barger 2002, Sandidge 2004) and in close association to conspecifics (Fischer and Vasconcellos-Neto 2005). As shown by the South American species, *Loxosceles gaucho* Gertsch, this may be, in part, due to species-recognition in female–female (Stropa and Rinaldi 2001) and male–male interactions (Stropa 2007) that either minimizes aggressive encounters and/or initiates early escape to avoid predation. Many *Loxosceles* spiderlings can be reared to subadulthood in a single jar without cannibalism as long as there is sufficient insect prey for food and crevices for hiding spaces (Vetter and Rust 2008). In

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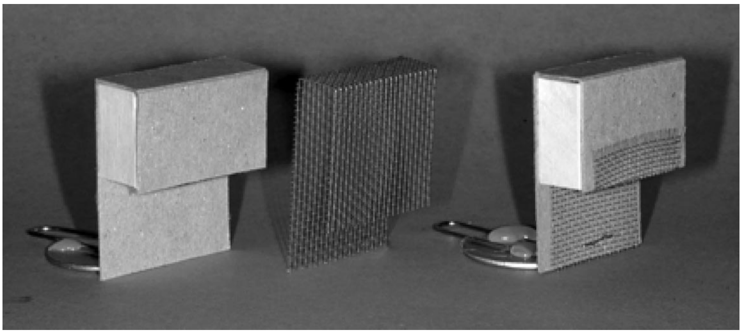

Fig. 1. Refugia and inserts used in experiments. Left, cardboard refugium with wood side spacers and 15- by 42-mm cavity, held upright by a bent paper clip affixed to a metal washer. Center, inverted, J-shaped aluminum screen on which silk was deposited for the solvent extraction tests. Right, refugia with a screen inserted into the cavity and affixed in place with a staple and bent edge of the screen.

a laboratory study regarding refugia preferences of *Loxosceles* spiders (Vetter and Rust 2008), *Loxosceles reclusa* Gertsch and Mulaik and *Loxosceles laeta* (Nicolet) preferred to inhabit small cardboard refugia in which a conspecific had been housed compared with never-occupied refugia. It is unknown whether this preference was chemical (pheromonal), physical (presence of silk), some other factor or a combination of variables. Nothing is currently known about *Loxosceles* pheromones, either for sexual communication or aggregation behavior.

Because of the toxic nature of *Loxosceles* bites, there is a desire to control these spiders in homes throughout much of the heavily human-populated areas of the Western Hemisphere where the spiders exist. Emphasis in this study focused on testing if *Loxosceles* preference for previously inhabited refugia was species specific, physically and chemically mediated, and restricted to taxonomically close species. We investigated this refugia preference in hopes of using this information for the development of control methods for these medically important spiders.

Materials and Methods

General Methods. Details of the origin of the spiders and methods are reported in Vetter and Rust (2008). In brief, cardboard refugia were set up in round plastic arenas and spiders were introduced at sunset such that they were allowed until the next morning to investigate the arena and choose a diurnal resting place. Spiders were offered a pair of refugia to determine whether there was preference for the parameters we attempted to manipulate. Differences from previous work are explained herein.

In all studies, cardboard refugia with wood side spacers (Fig. 1, left) were similar to those in Vetter and Rust (2008) except that they were of uniform crevice width (15 mm), with dimensions of 50 mm in height on the long side, 42 mm in width, and a cavity of 25 mm in depth. The 15-mm crevice-width size was near the midrange of size preference for the two species (Vetter and Rust 2008); hence, 15 mm was chosen as the standard. These dimensions allowed upright placement of the cardboard refugium inside a 40-dram plastic vial (48 mm in diameter, 85 mm in height) for silk deposition. In a vertically oriented refugium, the access opening to the crevice was at the bottom of the cavity such that the spider had to crawl up into the refugium to hide (Fig. 1, left, right). All spiders were maintained and tested in a room with west-facing windows covered with unwaxed, brown paper; therefore, they received diffuse light of a natural light cycle at 33° 58′ N (photoperiod of 15:9 [L:D] h at summer solstice), strongest in the afternoon. Room temperature varied from 22 to 28°C. Orientation of refugia was consistent within a single arena such that access to the downward-facing opening was either from a clockwise or counterclockwise direction; the two refugia were further aligned in north-south or east–west orientations. Orientations were alternated among the four possible combinations (clockwise or counterclockwise combined with north–south or east–west) along a row of arenas within an assay (see Vetter and Rust 2008 for a more detailed description and a figure of the orientation).

All spiders (silk donors or "chooser" spiders) were offered food 3 d before being used in experiments. In general, the types of spiders being offered food showed differing propensity to feed; after 30 min from introduction of a German cockroach, *Blattella germanica* (L.), of appropriate size (i.e., approximately the same body length as the spider) to each container, feeding was typically observed in all immatures, approximately half of the females and only a few males. Lack of feeding in females was probably due to satiation from prior feedings as most had distended abdomens; adult *L. laeta* females can survive more than a year without food before succumbing (Lowrie 1980). In contrast, male abdomens were rarely distended and reticence to feed occasionally resulted in death of some males during trials where no food was offered for 4 wk. We have no idea whether these group feeding differences affected the silk production in the assays. Most importantly, immatures were only chosen if they fed; this prevented us from choosing a spider preparing to molt because many spiders, including *L. reclusa,* stop feeding for approximately a week before

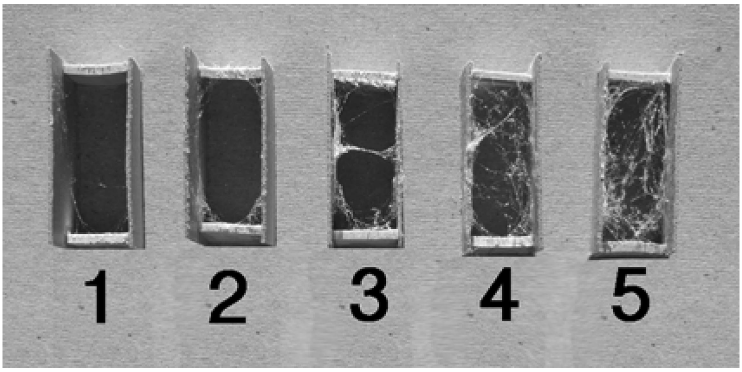

Fig. 2. View looking straight into the cavities of silk-covered refugia showing silk ratings of one (lowest) to five (highest).

molting (R.S.V., unpublished data). For all assays, spiders were chosen from those observed feeding, although nonfeeders with distended abdomens from previous feedings (mostly satiated females) also were chosen to reach the necessary total when there were not enough active feeders before an assay.

In preliminary tests, we noticed that *L. laeta* spiders deposited more silk in the refugia than did *L. reclusa.* This is due, in part, to *L. laeta* being a larger and stockier spider. Anecdotally, *L. laeta* consistently deposited more silk in the maintenance vials, so there also may be species differences in silk deposition in addition to size differences. Therefore, after a silk deposition period, each refugium was given a silk rating from 1 to 5 (Fig. 2): 1, no silk to just a few visible strands; 2, visible strands but no significant coverage; 3, visible silk on flat surfaces and also may include a few strands across the crevice opening; 4, significant silk deposition on all flat surfaces with significant silk stretched across the crevice opening; and 5, heavy silk deposition on flat surfaces and across the crevice opening. Silk ratings were assigned to each refugium and are based on both silk deposition by the spider and silk remaining after the spider was carefully flushed out of the cavity with a probe (i.e., a crevice may have originally been a rating of a 4 but reduced to a three after spider removal). Initially, the designator of the silk rating was not blind to identity of the spiders and refugia but adhered as strictly as possible to the categories mentioned above. In later trials, the refugia were randomized by a second person before the designator blindly assigned category numbers. However, considering the large number of mixed refugia that lay before the designator, it was highly unlikely that lack of being blinded to the origin had any influence on the designation.

We realize that this 5-point rating system has its deficiencies (i.e., qualitative data determined by a human observer), but other systems such as weighing refugia before and after likewise have deficiencies (i.e., weight gain [or potential loss in the cases of drastic humidity differences] cannot be attributed solely to the addition of silk); for the purposes of discerning the level of silk a spider may encounter upon entering a silk-covered refugium, we felt the 5-point system worked sufficiently. As pointed out in the review process, a quantitative measure would have provided nonbiased information; however, in the end, it is somewhat of a moot point because the amount of silk did not have nearly as much influence as we had initially hypothesized.

A bent paper clip with a metal washer affixed with hot glue (Craftsman all-purpose round glue sticks, 80453, Sears and Roebuck, Hoffman Estates, IL) was taped to the back surface of each refugium (Fig. 1, left) to support the refugium in the upright position and placed in a round, clear polystyrene container (185 mm in diameter by 76 mm in height) (see fig. 2 in Vetter and Rust 2008). The glue was affixed several days before testing and stored at room temperature on a countertop to evaporate odors. After the test was terminated, metal washers were removed from paper clips, washed in acetone, and dried for reuse. Refugia and paper clips were discarded, and arenas were washed with warm water and paper towels.

Even though recordings of the presence of spiders in refugia were made for 4 d during testing, statistics were performed on the day 1 data because this represented the event where each spider made a decision under the most uniform experimental conditions across the cohort, i.e., without the influence of its own experience or own silk in any one refugium.

Species Specificity of Silk Preference. Herein, bioassays were performed to determine whether *Loxosceles* spiders differentiate between refugia occupied by their own species versus that of a congeneric and their own versus a distantly related spider that makes similar type silk.

Congeneric Preference. On day 1, ≈30 spiders of both *Loxosceles* species were offered one German cockroach. On day 4, 20 silk-donor spiders of each species were placed in individual 40-dram vials with a cardboard refugium. On day 15, 20 chooser spiders of each species were each offered a German cockroach. On day 18, the 40 silk donors were forced from their cardboard refugia. At dusk of day 18, 10 *L. laeta* and 10 *L. reclusa* chooser spiders were introduced into individual arenas containing a refugium from each *Lox-*

osceles species. Five replicates of this experiment were performed.

Refugia choice based on species and silk ratings between species were analyzed using an R × C test of independence and a Kruskal–Wallis one-way analysis of variance (Sokal and Rohlf 1969).

Comparison to Distantly Related Species. We tested the ability of *Loxosceles* spiders to differentiate between conspecific silk and that of a distantly related spider; if *Loxosceles* spiders are not overly selective for silk preferences, it could be easier to produce silk attractant for control measures. Cribellate spiders, *Metaltella simoni* (Keyserling) (Amphinectidae), of body length 7–10 mm, were collected under trash cans and rotting wood around southern California (Riverside, San Diego, and Laguna Beach). Although *Loxosceles* spiders are ecribellates, their silk has similar properties to cribellate silk (Knight and Vollrath 2002).

On days 1 through 4, *M. simoni* spiders were each offered German cockroaches of similar or smaller body size. Multiple feedings were necessary because *Metaltella* spiders are more prone to starvation than *Loxosceles* spiders; the 2-wk silk donor period without food was estimated to be the safe limit of withholding food from *Metaltella* without fatalities (R.S.V., unpublished data). Spiders were not offered food during the silk donor period because this would have added a variable not present in the previous assays (i.e., a fed-upon carcass). A small piece of moistened cotton was held in place by the snap-on lid because *Metaltella* prefer more humid conditions than do *Loxosceles* spiders; the *Metaltella* spiders also were kept under a dark cloth. On day 4, the silk-donor *M. simoni* spiders were transferred into individual 40-dram vials containing cardboard refugia. All *Metaltella* spiders hid inside the darkened crevices similar to the *Loxosceles* spiders. On day 15, *Loxosceles* spiders were offered a German cockroach; one series was run with 12 *L. reclusa* and a separate series with 17 *L. laeta.* The sample size differed because of the availability of *Metaltella* spiders at the time of testing. On day 18, the *Metaltella* spiders were forced from their refugia, the latter of which were given silk ratings as listed above. The *Metaltella* refugia were paired with conspecific *Loxosceles* refugia with similar silk ratings which had been used in previous experiments and had not been occupied for >2 mo. Refugia were set up as before in the pairwise comparison protocol listed above. At dusk of day 18, *Loxosceles* spiders were introduced into the arena.

Refugia choice based on species of silk donor was analyzed using an R × C test of independence (Sokal and Rohlf 1969).

Chemically Mediated Preferences. Herein, we attempted to determine whether there were chemical components of the silk influencing preferences. In these two assays, only *L. laeta* spiders were used as silk donors and chooser spiders; *L. reclusa* was not used because of low silk production and insufficient number of adult silk donors.

Comparison of Silk Age and Heating. We compared refugia that were freshly removed from spiders to refugia that had been taken from the same batch of spiders 2 wk prior and subjected to heating. We assumed that if volatile chemicals were involved in the preference, that exposure to heat as well as aging would drive off volatiles and reduce their capability to influence spider behavior. This was based in part because in jumping spiders, male response diminishes or abates as silk is aged over 1 wk to 1 mo (Jackson 1986, Clark and Jackson 1995).

On day 1, 30 mature *L. laeta* spiders were each offered a German cockroach. From these, 10 males and 10 females were chosen as silk donors of which only 18 were used. On day 4, these 20 spiders were individually placed in 40-dram vials with a cardboard refugium. On day 18, silk donors were forced from refugia and replaced in the same vial with a second clean cardboard refugium. The silked refugia that were removed were separated by sex of the former occupant. The order for the first replicate was determined by a coin toss; male-silk refugia were placed into a hot plate oven (Thermolyne OV-10600, Barnstead Thermolyne Corp. Dubuque, IA) at 60°C for 4 d, whereas the female refugia were held in the assay room at 22 to 28°C. Spider silk is remarkably stable from −60 to 150°C (Yang et al. 2005). On day 22, the male-silk refugia were removed from the oven and placed in the assay room at 22°C. The oven was run for 1 h then turned off and the door opened to vent for the next 72 h. On day 25, the female-silk refugia were placed in the oven at 60°C for 4 d and then removed and stored at 22°C. The order for heating was alternated in the subsequent four replications of the assay. We had no a priori knowledge of whether volatiles were present, whether they are used for communication in *Loxosceles* spiders and, if present, whether the 60°C treatment would eliminate them.

On day 29, 30 additional *L. laeta* spiders were each offered a German cockroach, from which 18 chooser spiders (six each of males, females and subadults) were taken. On day 32, silk donors were forced from the second set of refugia. To minimize variation in silk characteristics, the pair of refugia (one aged/heated, one freshly vacated) within an arena originated from the same silk-donor spider. All silk-coated refugia were given silk ratings as described above.

Ten hours later at dusk of day 32, one *L. laeta* chooser spider was introduced into each of the 18 arenas. Because each assay required the use of 54 spiders (36 as donors, 18 as choosers), the limited number of available spiders (especially males who would feed and could survive the 4-wk silk donor starvation period) required that some were used in multiple trials. This assay was performed five times.

Refugia choice data based on treatment for day 1 data and silk ratings between sexes of *L. laeta* were analyzed using an χ^2 test and a Wilcoxon-signed-rank test (Sokal and Rohlf 1969), respectively.

Effect of Solvent Washing of Silk. Silk was submerged in solvents in another attempt (using a different technique than heat) to determine whether semiochemicals played a role in preference. A range of solvents sufficient to remove a wide array of polar to nonpolar compounds included water, acetone, hexane, and a 2:1 mixture of methylene chloride:methanol. Each replicate consisted of silk derived from 20 spiders.

To facilitate solvent washing of silk, commercially-available aluminum screen (5.9 by 7.1 wires per cm) was cut into 100- by 35-mm pieces, washed in acetone for 30 s to remove possible contaminants, and then air-dried. Screen was folded into a square-bottomed J-shaped insert and inverted (Fig. 1, center); this was slid into the crevice of the refugium. The folded screen covered the flat cardboard surfaces of the crevice but not the side wooden spacers. To hold it in place, the screen was stapled near the bottom on the long back surface and folded upwards on the short front surface (Fig. 1, right).

On day 1, 15 immature and 15 female *L. laeta* were each offered a German cockroach; from these, 10 of each were chosen as silk donors. On day 4, the 20 silk-donor spiders were placed in individual vials with each refugium fitted with an aluminum screen. On day 15, an additional 15 female and 15 immature *L. laeta* were each offered a German cockroach; from these 10 of each were selected as the chooser spiders.

On day 18, screen inserts were removed from the retreats, flattened and immersed, two at a time in 25 ml of solvent (20 ml for the less volatile water) in a glass petri dish (88 mm in diameter by 20 mm in height) for 2 min. All 10 screens from either females or immatures were eventually immersed in the same solvent bath. After 10 min of total immersion (two screens by five bouts of 2-min immersion), ≈15 ml of solvent remained; 1.5 ml of solvent was then applied to each of 10 new refugia. Control refugia had 1.5 ml of clean solvent applied to each. All refugia were placed on their sides in an exhaust hood to dry. Solvent washing and application occurred before noon so ≈8 h transpired before introduction of refugia to the test arenas. On the evening of day 18 at dusk, 20 chooser spiders were individually introduced into arenas with a solvent wash refugia and a control in four combinations: five females were offered refugia with solvent from immature spider washes, five females were offered female washes and likewise for the 10 immatures. This bioassay was performed once for water, hexane, and 2:1 methylene chloride:methanol extracts due to no statistical preference. However, because the first acetone extract showed disparate choice results, this assay was performed five times.

Extract data were analyzed using an R × C test of independence and chi-square tests for control versus extract; in addition, for the acetone experiment, the female versus juvenile choosers and female versus juvenile extract (Sokal and Rohlf 1969).

Results

Species Specificity of Silk Preference. *Congeneric Species.* In this experiment, neither *Loxosceles* species preferentially chose refugia in which conspecifics had laid down silk ($G = 0.85$, df = 1, $P = 0.35$; $N = 99$) (Fig. 3). *L. laeta* had significantly higher silk ratings for crevices that they inhabited for 2 wk (median = 4, mode = 4) compared with *L. reclusa* (median = 3, mode = 3) ($G = 56.07$, df = 5, $P < 0.0025$; $N = 99$ for each species) (Fig. 4).

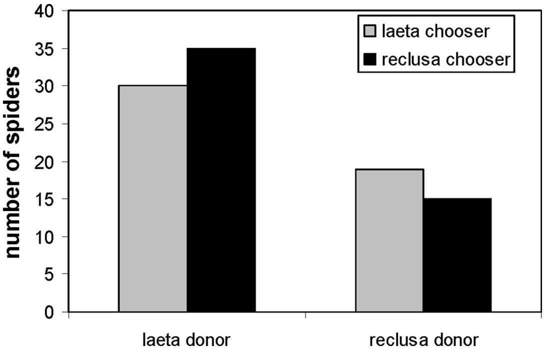

Fig. 3. Choices of *L. reclusa* and *L. laeta* spiders for silk-covered refugia occupied by a conspecific or the other species.

Distantly Related Species. The *Metaltella* silk ratings ranged from 2 to 4, and the silk was often deposited across the opening of the crevice; however, the fibers did not seem as thick as *Loxosceles* silk. After the first night in the experimental arenas, both *Loxosceles* species chose refugia inhabited by conspecifics in comparison to refugia from which *Metaltella* spiders had recently been evicted (*L. reclusa:* $\chi^2 = 8.33$, df = 1, $P < 0.01$; $N = 12$; and *L. laeta:* $\chi^2 = 15.00$, df = 1; $P < 0.01$; $N = 17$) (Fig. 5). For *L. reclusa,* 11 of 12 were only found in refugia previously occupied by conspecifics over the 4-d observation period; the one outlier was found in the *Metaltella* refugium for three of the four morning checks and in the conspecific refugium the third night. During the 4-d trial, 13 of 17 *L. laeta* were found exclusively in the conspecific silk refugia; two spiders chose the *Metaltella* silk refugium for one of the four observations, another for two observations and one spider was found in the non-*Loxosceles* refugium each time. To summarize, over the 4-d observation period, *L. reclusa* spiders were only found in *Metaltella* refugia in three of 48 (6%) times and *L. laeta,* eight of 68 (12%) times.

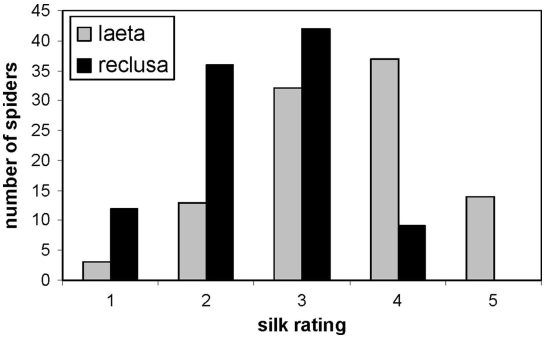

Fig. 4. Silk ratings for refugia occupied by *L. reclusa* and *L. laeta* spiders for 2 wk.

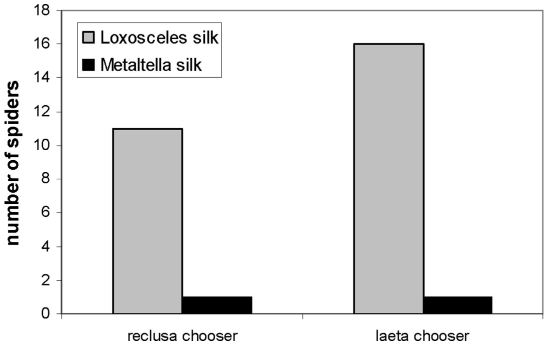

Fig. 5. Choices of *L. reclusa* and *L. laeta* spiders for silk of their own species or that of a distantly related cribellate spider *M. simoni.*

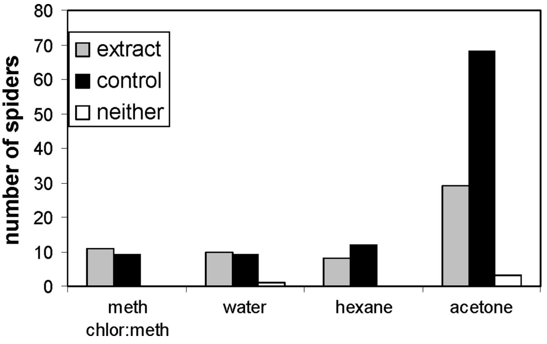

Fig. 7. Choices of *L. laeta* immatures and females for various solvent extracts of silk from immature and female conspecifics.

Chemically Mediated Preferences. *Comparison of Silk Age and Heating.* When *L. laeta* spiders were offered a choice between refugia that were freshly removed from silk donors or refugia that were 2 wk old and heated at 60°C for 4 d, they chose the older refugia 56 times versus 34 for the freshly vacated refugia. This difference was statistically significant ($\chi^2 = 5.38$, df = 1, $P < 0.05$; $N = 90$).

In this assay, *L. laeta* females received significantly higher silk ratings (median = 3; mode = 3) than did *L. laeta* males (median = 2; mode = 2) ($H = 87.44$, $P < 0.0001$; $N = 90$ for each gender) (Fig. 6). There was no statistical difference in silk rating produced by the spiders for the refugium occupied during weeks 0–2 compared with weeks 2–4 for males (first week median = 2; second week median = 2; $T_s = 0.032$, $P = 0.974$) nor females (first week median = 3; second week median = 3; $T_s = 0.019$, $P = 0.985$).

Effect of Solvents. When females and mid-sized juveniles were offered retreats treated with silk extracts, they showed no preference for these solvents (water: $\chi^2 = 0.2$, df = 1, $P > 0.05$; $N = 20$; hexane: $\chi^2 = 0.8$, df = 1, $P > 0.05$; $N = 20$; and 2:1 mixture of methylene chloride:methanol $\chi^2 = 0.2$, df = 1, $P > 0.05$; $N = 20$) (Fig. 7). However, when acetone was used for the extraction, spiders chose the control refugia significantly more often than they did the acetone extract-treated refugia ($\chi^2 = 15.68$, df = 1, $P < 0.001$; $N = 100$). For the acetone trials, there were no statistical differences between the choices made by the 50 females compared with the 50 juvenile spiders ($\chi^2 = 0.75$, df = 3, $P = 0.387$), and the repellency of the pooled 50 female silk extracts was similar to pooled 50 juvenile silk extracts ($\chi^2 = 0.54$, df = 3, $P = 0.464$).

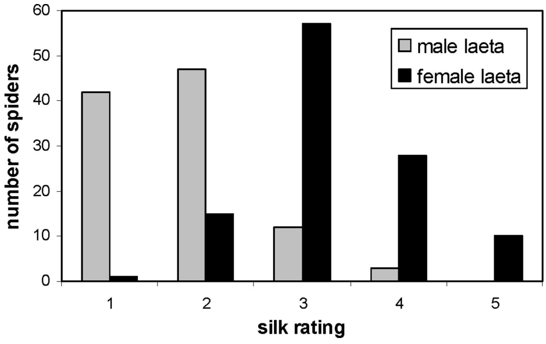

Fig. 6. Silk ratings for refugia occupied by male and female *L. laeta* spiders for 2 wk.

Discussion

Factors involved in conspecific preference of *Loxosceles* spiders for previously inhabited refugia have been investigated herein. There was no species specificity exhibited by *L. reclusa* or *L. laeta.* However, *L. laeta* spiders preferred heated, aged refugia compared with freshly vacated refugia. There was no preference to any of the silk extracts, but there was the unexpected result that silk extracted with acetone was significantly repellent to *L. laeta* spiders. Because there was no positive preference to any chemical manipulation that we offered, the attraction by *Loxosceles* spiders for refugia previously inhabited by conspecifics as shown in Vetter and Rust (2008) is most probably due to the physical presence of silk and, hence, can accurately be termed sericophily.

For recluse spiders, the choice of a refugium that already is lined with conspecific silk (Vetter and Rust 2008) might indicate that this was an acceptable place to seek a safe harborage. Levi and Spielman (1964) noted that removal of a *L. laeta* female was followed by the sequential occupation by immatures later during the week; the attraction could have been the silk, the protective nature of the retreat, a combination of these two or possibly other factors. In addition, the presence of *Loxosceles* webbing would mean that the new occupant would not have to expend proteins in laying down additional silk for a retreat. However, the preference for older refugia and the repellency of acetone-washed silk might indicate that there are semiochemicals that indicate the recent occupancy of a conspecific. Because *Loxosceles* spiders show strong site fidelity as is evident by frequent cases of multiple shed skins of increasing size at a particularly site, a freshly abandoned, silk-covered retreat could be a temporary vacancy and, hence, avoidance by an interloping conspecific would reduce agonism. Similarly, both *Loxosceles* species showed a strong preference for conspecific silk compared with that of *Metaltella simoni;* avoidance of a non-*Loxosceles* silked retreat could reduce predation risk from other spider species.

The aspects determining *Loxosceles* sericophily presented here may be evident in studies involving other species. The araneid orb weaver *Zygiella x-notata* (Clerck) prefers to construct webs where conspecific silk and preexisting retreats occur (Leborgne and Pasquet 1987). When web-building spiders (*Hypochilus thorelli* Marx [Hypochildae] and *Parasteatoda* (=*Achaearanea*) *tepidariorum* (C. L. Koch) [Theridiidae]) were removed temporarily from their webs on rock faces, they preferred to move into uninhabited webs rather than build their own (Hodge and Storfer-Isser 1997). They also demonstrated that when spiders were removed from a home outcrop and released at an outcrop with inhabited webs, they preferred to take over an inhabited web and oust the occupant or they built a new web on the periphery of one of the inhabited webs. Because there was no correlation to web site selection and its environmental characteristics such as temperature, height, humidity, or angle of rockface, Hodge and Storfer-Isser (1997) concluded that this aggregation behavior was due to behavioral (i.e., sericophilic) mechanisms.

Our study has several implications in regards to pest management strategies. The avoidance of refugia with fresh silk and the chemical repellency of the acetone wash warrant further study. If the repellent component(s) can be identified, it could be an effective control for *Loxosceles* spiders if sprayed around a home or in areas of concern such as the legs of a baby's crib. It did not matter whether the source of the silk was from females or juveniles or whether female and juvenile spiders were making the choice; responses were similar within each set of parameters. Therefore, repellency is not limited to one life stage such that control may be possible throughout the entire life cycle of the spiders.

Because *Loxosceles* spiders prefer harborages with congeneric silk (Vetter and Rust 2008), traps with artificial silk deposits might be developed. In addition, *Loxosceles* spiders do differentiate between conspecific silk and that of a distantly related spider so any control device would need to carefully replicate the physical features of *Loxosceles* silk to maximize attractancy. However, there is nothing that looks overtly promising at this point. Although the information provided herein does not suggest chemical attractants which could easily be manipulated for pest control measures, one positive aspect is the lack of species specific responses to each other's silk. Considering that these two *Loxosceles* species are separated widely both geographically and, within the genus, taxonomically, something might develop for control throughout the genus. Finally, if attempts are made to extend this research, future researchers should keep in mind the silk production differences between the species (Fig. 4) and between the sexes (Fig. 6) shown herein when choosing their silk donors.

Sanitation is considered important for the long-term control of spiders in and around structures, especially web-building spiders (Vetter 2010). In the pest control industry, insecticide application is primarily directed at spiders, at surfaces where spiders may construct webs, and at cracks or crevices where they might harbor (Vetter 2010). However, in one study, silk treated with fipronil or cyfluthrin killed significantly more widow spiders, *Latrodectus hesperus* Chamberlin and Ivie, and hobo spiders, *Tegenaria agrestis* (Walckenaer), than did similar surface applications (Gaver and Hansen 2005). This suggests that spiders may be more readily controlled by treating the silken webs. Residual deposits (1 d old) of permethrin were not repellent to a number of hunting and web-building spiders (Pekár and Haddad 2005).

As we have shown herein, *Loxosceles* attraction to silk or the repellency to some silk extract may aid in the control of this medically important spider genus. The attraction of *Loxosceles* to congeneric silk suggests that sprays be applied to webbing in one process of treating cracks and crevices maybe highly effective in controlling them.

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